INTERCELLULAR JUNCTIONS, MOTILITY AND MAGNETOSOME STRUCTURE IN A MULTICELLULAR MAGNETOTACTIC PROKARYOTE

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ABSTRACT

A many-celled, magnetotactic, prokaryote obtained from brackish water possessed intercellular connections at points of contact between the outer membranes of constituent cells. These connections structurally resembled the "gap junctions" found in eukaryotes. Each aggregate organism consisted of 10 to 30 individual gram-negative cells containing material with the appearance of poly-ß-hydroxybutyrate and magnetosomes of unusual arrangement, structure and composition. The aggregate, which possessed prokaryotic-type flagella arranged at the outward surfaces of each cell, showed motility indicative of coordination between individual component cells. These results suggest that this organism is a multicellular prokaryote.

INTRODUCTION

Magnetotactic bacteria orient and migrate along geomagnetic field lines (Blakemore,1975 and 1982). Many morphologically diverse types inhabit freshwater and marine waters and sediments (Blakemore et al., 1989). An unusual magnetotactic microorganism has been collected from sulphide-rich marine and brackish nearshore, pond, and lagoon waters and sediments of the east and west coasts of North America. The organism was a highly motile, highly refractile spherical cell aggregate or microcolony of prokaryotic cells that migrated as an intact unit in the geomagnetic field direction. A similar organism that migrated opposite to the geomagnetic field direction has been collected in Brazil (Farina et al., 1983).

Fossil records of the first two billion years of life on Earth reveal that the first cells identifiably preserved in rocks were prokaryotic in nature (Schopf and Walter, 1983; Schopf and Packer 1987). The evolution of eukaryotes commenced about 1.4 billion years ago (Vidal, 1981) and was associated with increases in the size and complexity of organisms. Many extant eukaryotic species are multicellular and possess a tissue form of organization the integrity of which is maintained by specialized intercellular connections. Such junctions serve as low resistance pathways for ion conduction and coordinate function among cell groups. We report here on the structure and motility of this many-celled magnetotactic organism. Of particular interest is the observation that individual cells in the aggregate organism are connected to each other by outer membranes that join together forming regions with the structural appearance of cell-to-cell junctions. Our findings are consistent with the possibility that multicellularity involving intercellular connections in prokaryotes predated that in eukaryotes.

MATERIALS AND METHODS

Organism collection

Glass jars filled with black mud or sand and water from marine and brackish coastal sites in New England with salinity values between 12 and 32 parts per thousand were stored loosely covered in dim light at room temperature (25°C) and left undisturbed for several days. Rusty-colored films at the air/water interface and the odor of H_2S were characteristic of these natural enrichments. At this time, pH values of the water were usually 7.3 at the surface and 6.5 near the bottom where the magnetotactic bacteria were located. Total iron values in surface sediments, as measured by the ferrozine method (Stookey, 1970), ranged from a low of 0.6 to as high as 28.7 mg/liter. In contrast to other types of magnetotactic bacteria, these aggregate organisms usually disappeared from the enrichments within three weeks, at which time large populations of photosynthetic green sulfur bacteria appeared. Suspensions of organisms for phase contrast and electron microscopy were harvested by applying a magnet to the outside of the jars (Moench and Konetzka, 1978). Unlike other magnetotactic bacteria which aggregate in dense cell pellets, these many-celled organisms localized in a loose cloud at the south magnetic pole.

Electron microscopy

Organisms in suspension were prepared for transmission electron microscopy by fixation in 3% (v/v) glutaraldehyde in 0.05M cacodylate buffer containing 10mM magnesium sulphate at pH 7.3 (Rodgers and Davey, 1982). The fixed suspensions were centrifuged and the pellets preembedded in 2% (w/v) agarose for further preparation for thin-section electron microscopy. Samples were post-fixed in 1%(w/v) OsO_4, dehydrated in a graded ethanol series and embedded in an Epon-araldite resin mixture. Thin-sections were cut on an LKB Ultratome III, stained with 5% (w/v) uranyl acetate for 60 sec and 0.4% (w/v) lead citrate for 20 sec (Rodgers, 1979). Similar organism suspensions were applied to formvar-carbon-coated grids and negatively stained with 2.5% (w/v) ammonium molybdate. Thin-sectioned and negatively stained material were examined in a Hitachi H600 or Jeol 100S electron microscope. For scanning electron microscopy, organisms were critical point dried from CO_2, sputter coated with gold and examined in an AMR 1000 scanning electron microscope. Thin-sections were also examined by energy dispersive X-ray analysis in a VG Microscopes HB 5 scanning transmission electron microscope fitted with a field emission electron source.

Magnetotaxis

The permanent magnetic dipole moment of each of 5 intact organisms was estimated by measuring the average bacterial migration rates in the presence of applied magnetic fields ranging from 0.7 to 5.8 gauss and fitting the data to a Langevin function (Kalmijn, 1981). The effect of a demagnetization procedure on swimming bacteria was evaluated using a commercial 60Hz AC degausser with peak fields of several hundred gauss.

RESULTS AND DISCUSSION

Motility and Structure

The multicellular organisms were spherical, approximately 12.5 μm in diameter with a rosette or mulberry-like morphology (Fig. 1a and b). Smaller forms, 3 to 8 μm in diameter, with similar morphology were also observed. The organism displayed a coordinated rolling or spinning motility with effective translational movement in the magnetic field direction. Swimming speeds as assessed on 35 of the multicellular organisms ranged from 67 to 175 (average 105) $\mu m.sec^{-1}$ in a uniform magnetic field of 0.7 gauss. At the edge of an uncovered water droplet the organisms made intermittent excursions in the reverse of the field direction for about 100 to 500 μm at swimming speeds approximately twice the forward speed, then returned to the edge of the drop at normal forward speed. The significance of this "ping-pong" motion, apparently peculiar to this organism, is unclear. Organisms trapped at the water-air interface were observed to rotate rapidly without translation. In open water droplets the organisms rapidly became non-motile. Exposure of the intact aggregate organisms to low osmotic pressure using distilled water caused a loss of motility and eventual disruption into clusters of unattached non-motile cells (Fig. 1c). These consisted of 10 to 30 roughly ovoid cells each approximately 0.8 to 1.4 µm in length and 0.6 to 0.8 µm in width. Restoration of the original osmolarity did not restore aggregate structure or cell motility.

Flagella on the organism were of the unsheathed prokaryotic-type, each 21.5 nm in diameter and located on the outer free surfaces of constituent cells (Fig. 1d). Forward thrust necessary for translation of the organism could have been provided by formation of a flagellar bundle or bundles, or by flagellar rotation in one or a few selected cells. The excursions opposite to the imposed constant (DC) magnetic field direction after collision with the edge of the water droplet could have been a tactic response in which the organism reversed the direction of flagellar rotation for a short time. If a flagellar bundle were involved, such reversed rotation might be expected to cause a flagellar bundle to fly apart with loss of translational movement, contrary to the behavior observed by phase contrast microscopy. If several constituent cells were responsible for motility of the organism, reversal of the swimming direction could have involved coordination or communication between them.

In thin-section, each cell in the aggregate was structurally prokaryotic and gram negative in nature. Each possessed a cytoplasm rich in dispersed ribosomes with a fine skein of nuclear filaments, electron dense particles and granules with the appearance of poly-ß-hydroxybutyrate (PHB) (Fig. 1e). These granules appeared black on staining for light microscopy with Sudan Black B. The bacterial cytoplasm was limited by a plasma membrane with an outer membrane enclosing a periplasm with little evidence of peptidoglycan. The cell outer membranes were tightly apposed at the interior of each of the organisms (Fig. 1f). The apparent lack of rigidity of the murein layer in each cell would facilitate this process. These morphologically distinct, closely associated membrane regions had the appearance of membrane junctions, each of which were uniformly 2 nm in width and appeared to hold the individual prokaryotic cells together. It is possible that this tight apposition of membranes at these specialized junctions also allows intercell communication and could explain how individual cells participate in the coordinated motility observed for the entire organism.

Individual cells contained from 2 to 65 (average 31) electron dense particles occasionally arranged in rows (Fig. 2a and b). Each particle was irregular in shape and 85 to 90 nm in diameter. Those in parallel arrays were separated by an envelope and from adjacent particles by an amorphous region of 8 to 10 nm. X-ray mapping by energy dispersive elemental analysis showed that the bulk of the cell iron was located within these particles (Fig. 2c). Each particle contained a crystalline center of triangular or cuboidal outline measuring 40 to 45 nm in diameter in addition to a non-crystalline or amorphous halo 20 to 25 nm in width and of lower iron concentration (Fig. 2 d and e). Elemental analysis and electron diffraction of the smaller multicellular organism (approx. 6 µm in diameter) have shown that the crystalline particles consist of a mixture of the ferrimagnetic iron sulphide greigite, Fe_3S_4, and the nonmagnetic iron sulphide pyrite, FeS_2 (Mann et al., 1990), or pyrrhotite (Farina et al., 1990). Thus, the magnetosomes of the present organism appear to differ in chemical composition from those of magnetite (Fe_3O_4) containing magnetotactic bacteria (Frankel et al., 1979; Mann et al., 1984a and b; Matsuda et al., 1983).

FIGURE 1

Optical and electron micrographs of the multicellular magnetotactic prokaryote.

(a) Phase contrast photomicrograph of an intact organism in a wet preparation. Note rosette-like appearance of the multicellular prokaryote. (x 1,403).
(b) Scanning electron micrograph showing the distribution of flagella on the outer surfaces of the spherical shaped organisms within the many-celled prokaryote. (x 8,745).
(c) Phase contrast photomicrograph of a many-celled organism osmotically disrupted. Note individual cells with wide size distribution. (x1,238).
(d) Electron micrograph of a negatively stained individual cell of the multicellular prokaryote. Note asymmetric distribution of flagella. Magnetosomes and electron-lucent vacuoles resembling poly-ß-hydroxybutyrate (PHB) granules are evident. (x20,625).

(continued)

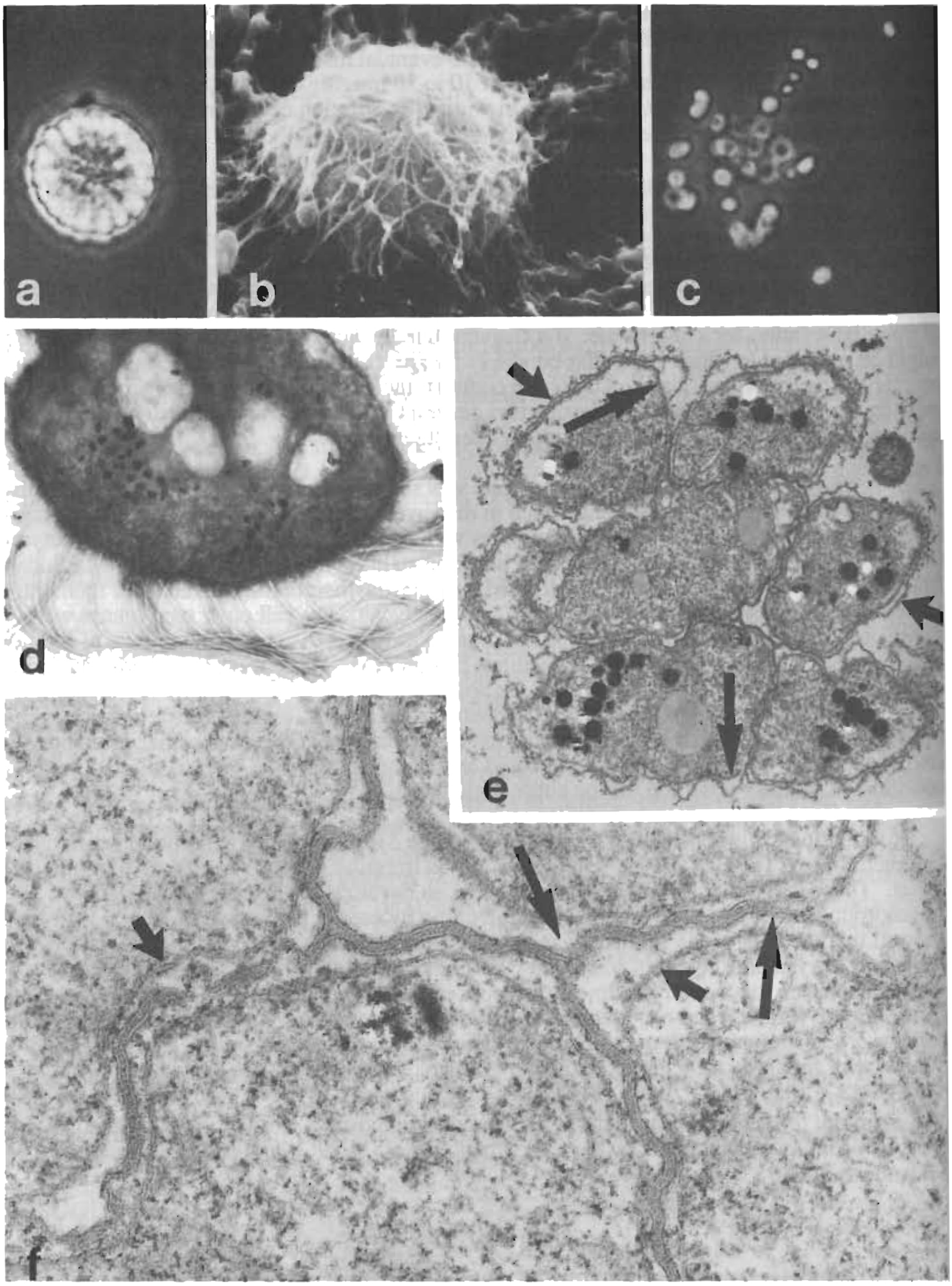

FIGURE 1 (Continued)

(e) Thin-section of aggregate organism illustrating gram-negative prokaryotic nature of each constituent cell. Note outer membranes (short arrows), cytoplasmic membranes (long arrows), magnetosomes and PHB granules. (x24,750).

(f) Higher magnification of contact regions between cells within the many-celled aggregate. Typical gram-negative type cytoplasmic membranes (short arrows) are evident. Note points at which juxtaposed intercellular outer membranes have fused at regions having a constant 2 nm width (long arrows). The crystalline nature of the bacterial magnetosome and the amorphous material surrounding it is evident. (x 92,070).

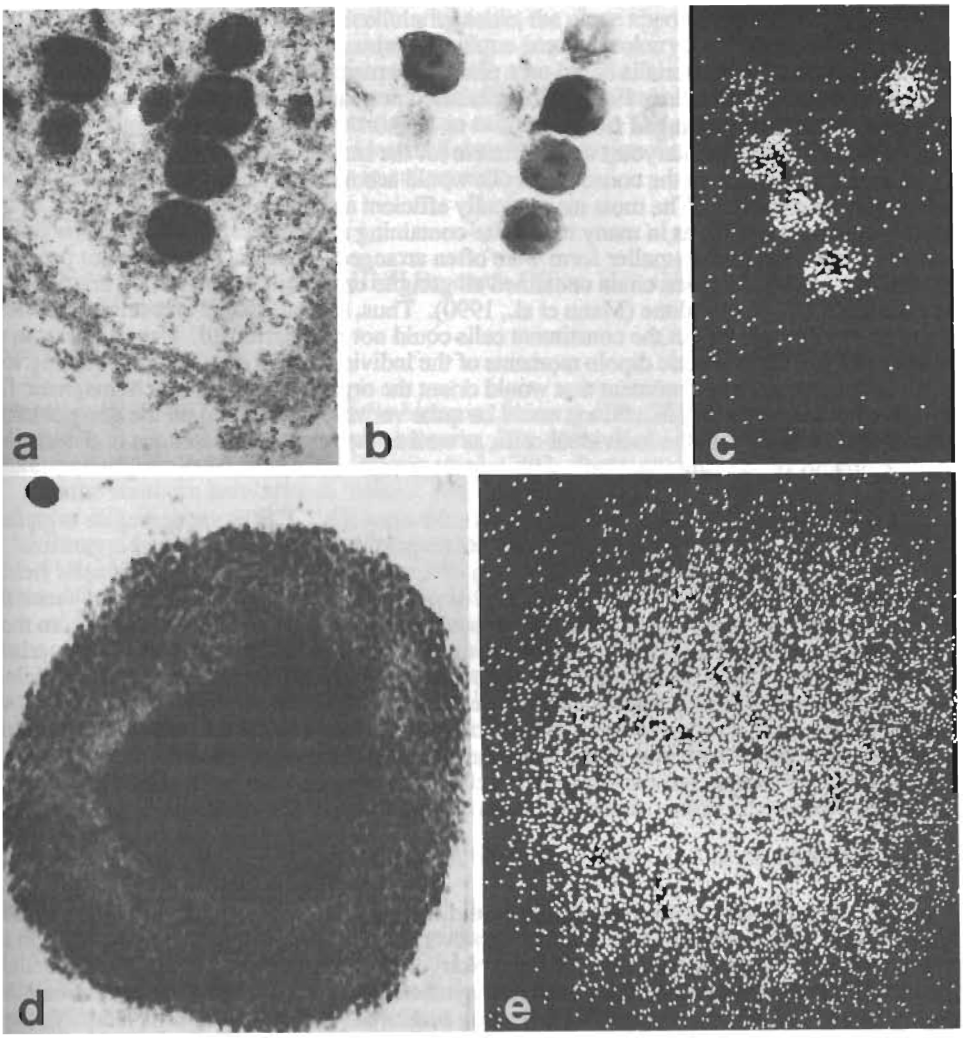

FIGURE 2

Thin-section electron micrographs and energy dispersive X-ray analysis (EDXA) of magnetosomes in the multicellular prokaryote.

(a) Short chain of magnetosomes present in the upper left bacterium of the multicellular organism shown in figure 1 (e). (x 93,885).

(b) Same micrograph printed lightly. Triangular or cuboidal internal sub-structure of individual magnetosomes and the amorphous zone surrounding each is shown. (x 93,885).

(c) Iron map of the area shown in figure 2 (a and b) using EDXA. White dots indicate the locations from which iron X-rays emanated. Note concentration of iron in regions corresponding to the magnetosomes. (x 93,885)

(d) High magnification of a single magnetosome showing a central triangular-shaped region and surrounding zone of lower electron-density. (x 845,000).

(e) Iron map of the area shown in figure 2 (d). Although iron was present in the entire magnetosome, it was concentrated preferentially in the central triangular or cuboidal regions. (x 845,000).

In addition, EDXA spectra produced from the amorphous and central zones of the magnetosomes as well as from the bacterial cytoplasm and background resin showed the highest concentrations of iron were found in the central "structured" regions of each magnetosome.

Magnetotaxis

Magnetotaxis in bacteria is based on a permanent magnetic dipole moment with a fixed orientation in the cell (Frankel, 1984). The measured permanent magnetic dipole moments of the aggregate organisms ranged from 5×10^{-13} to 1×10^{-12} erg · gauss cm^{-1} similar to those of other magnetotactic prokaryotes and sufficient for the magnetotactic response. The presence of ferrimagnetic greigite in the constituent cells would account for a permanent magnetic dipole moment in the organism. The most magnetically efficient arrangement would be chains of single magnetic domains, as in many magnetite-containing magnetotactic bacteria. The sulfide particles in the cells of the smaller form were often arranged in chains, but it was not possible to determine whether a given chain contained all greigite or all pyrite, or both, by inspection of the electron micrographs alone (Mann et al., 1990). Thus, the magnitude and orientation of the magnetic dipole moments in the constituent cells could not be determined. Nevertheless, in the intact organism, the magnetic dipole moments of the individual cells would add vectorally to give a net magnetic dipole moment that would orient the organism in the ambient magnetic field. Thus, the net magnetic dipole moment could be quite variable, depending on the greigite content and its distribution in the individual cells, as well as the relative orientations of the magnetic dipole moments of the individual cells with respect to each other in the intact organism.

Like unicellular magnetotactic bacteria (Blakemore et al., 1980), the aggregate organisms were remagnetized by exposure to the 60 Hz AC magnetic field. Remagnetized organisms subsequently migrated opposite to the direction of a weak (several gauss) DC magnetic field. Remagnetization implied that there was an axis of motility in the intact organism and hence the projection of the net magnetic dipole moment could be forward or reverse with respect to the direction of thrust (Frankel, 1984). However, unlike the unicellular magnetotactic bacteria (Blakemore et al., 1980), some aggregate organisms were demagnetized, subsequently failed to respond to changes in the direction of the weak DC field, and swam in seemingly random directions. Demagnetization of some organisms was consistent with relatively weak coupling of the magnetic dipole moments of the constituent cells in the intact organism; the demagnetization procedure probably left the constituent cells with permanent magnetic dipole moments, but reduced the vector sum of those moments to zero for the intact organism.

CONCLUSIONS

The magnetotactic aggregate organism studied here is morphologically similar to those collected in Brazil by Farina et al., 1983). However, intercellular connections like those in the present organism were not reported. Other prokaryotes known to exist as motile, multicellular aggregates include the myxobacteria and photosynthetic consortia. The former are unicellular gliding bacteria which aggregate to form fruiting bodies but with swarmer cells retaining their individuality throughout the process of cooperative morphogenesis. Photosynthetic consortia such as "Chlorochromatium" or "Pelochromatium" consist of dissimilar cells of several species and are not known to form specialized membrane adhesions. Intercellular connections have been reported in certain cyanobacteria (Lang and Fay, 1971) but these do not entail structures similar to those described here. As the cells comprising the present aggregate organisms are of the gram negative type, the closely apposed membranes involved are, by definition, outer bacterial membranes and not cytoplasmic membranes. Thus, these many-celled prokaryotes are like no other known organisms. The intercellular connections described here could be functionally important in the motility and magnetotaxis of the aggregate organisms, perhaps by coordinating flagellar activity among the constituent cells.

In eukaryotes, multicellularity refers to cell specialization and cooperation in a many-celled organism. While in prokaryotes, multicellularity has been less clearly defined, three requirements have been proposed (Starr and Schmidt, 1981): (a) the organism be many-celled, (b) the cells have a permanent and characteristic juxtaposition, and (c) the cells exhibit discernable distinction in structure or function. The organism reported here clearly satisfies the first two criteria. While no evidence has been found for cellular distinction in structure or function to support the final criterion, it may be argued that the complex motility exhibited by the intact aggregate but never by the individual cells alone, implies coordination of flagellar activity and communication between individual cells. Moreover, the asymetrical arrangement of flagella on each constituent cell may reflect a spatial distribution required for coordinated motility. We propose that the magnetotactic aggregate is a multicellular prokaryote.

However, definitions of multicellularity aside, the close apposition of the outer individual cell membranes in the intact organism is unique among prokaryotes. Furthermore, they provide the first example, in an extant prokaryotic species, of a fundamental morphological trait expressed among multicellular eukaryotes; that of intercellular membrane adhesions ultrastructurally similar in appearance to eukaryotic cell junctions. They provide evidence that multicellularity involving specialized intercellular membrane junction-like structures in prokaryotes may have preceded that in eukaryotes.

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